

which phenazine methosulphate is coupled to 2,6-dichlorophenol-indophenol. The pH optimum is 8.5 and the  $K_m$  for trimethylamine is about  $2\mu\text{M}$ . Of 42 compounds tried as substrate, the following gave an initial rate 10% or more of that with trimethylamine under the conditions tested: ethyldimethylamine, diethylmethylamine, 2-aminoethyldimethylamine, 2-hydroxyethyldimethylamine, 2-chloroethyldimethylamine and diethylamine.

The first step in the breakdown of trimethylamine by bacterium 4B6 thus appears to be the oxidative *N*-demethylation catalysed by trimethylamine dehydrogenase.

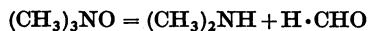
J. C. thanks the Science Research Council for a studentship.

- Andrews, P. (1965). *Biochem. J.* **96**, 595.  
 Eady, R. R. & Large, P. J. (1969). *Biochem. J.* **111**, 37P.  
 Schryver, S. B. (1910). *Proc. R. Soc. B*, **82**, 226.  
 Shapiro, A. L., Viñuela, E. & Maizel, J. V. (1967). *Biochem. biophys. Res. Commun.* **28**, 815.  
 Sze, Y. L., Borke, M. L. & Ottenstein, D. M. (1963). *Analyt. Chem.* **35**, 240.

### The Metabolism of Trimethylamine *N*-Oxide by *Bacillus* PM6

By P. A. MYERS and L. J. ZATMAN. (*Department of Microbiology, University of Reading, Reading RG1 5AQ, U.K.*)

*Bacillus* PM6 is an aerobic spore-forming Gram-positive rod-shaped organism isolated from soil. It is capable of growth on trimethylamine *N*-oxide, trimethylamine, dimethylamine or methylamine as sole source of carbon, nitrogen and energy. Washed suspensions of trimethylamine *N*-oxide-grown organisms rapidly take up oxygen in the presence of trimethylamine *N*-oxide, dimethylamine and methylamine, but not in the presence of trimethylamine. Such suspensions, and their cell-free extracts, convert trimethylamine *N*-oxide anaerobically into dimethylamine and formaldehyde, indicating the presence of a trimethylamine *N*-oxide demethylase. Assays of trimethylamine *N*-oxide (Bystedt, Swenne & Aas, 1959), dimethylamine (by g.l.c. and paper chromatography) and formaldehyde (Nash, 1953) yield results that agree with the stoichiometry required by the following equation:



In early experiments the reaction rate was followed by measuring formaldehyde production colorimetrically (Nash, 1953); subsequently a more sensitive and convenient assay was developed in which the trimethylamine *N*-oxide demethylase is

coupled to formaldehyde dehydrogenase (EC 1.2.1.1) (partially purified from baker's yeast) and the reaction is followed spectrophotometrically at 340nm.

The trimethylamine *N*-oxide demethylase was purified 175-fold. The purified enzyme moves as a single protein on analytical polyacrylamide-gel electrophoresis and has a molecular weight of 50000 as determined by gel filtration on Sephadex (Andrews, 1970). Treatment with 1% sodium dodecyl sulphate + 1% 2-mercaptoethanol caused no resolution of the protein as determined by polyacrylamide-gel electrophoresis (Weber & Osborn, 1969), suggesting that it consists of a single polypeptide chain; this experiment yielded a molecular weight of 37000. Ultracentrifugal analysis (sedimentation equilibrium) gave a molecular weight of 36000–47000. The pH optimum of the purified enzyme is 7.5, the  $K_m$  for trimethylamine *N*-oxide is 2.85mM and the absorption spectrum shows only a single peak at 280nm. Ferrous iron, glutathione and L-ascorbate are strongly stimulatory.

Benzyltrimethylamine *N*-oxide, chlorpromazine *N*-oxide and (+)-propoxyphene *N*-oxide were also active as substrate for the enzyme, but the maximum rates obtained were only about 5% of that obtained with trimethylamine *N*-oxide. Trimethylamine is a non-competitive inhibitor, and SKF 525-A is a competitive inhibitor.

The initial step in the metabolism of trimethylamine *N*-oxide by *Bacillus* PM6 thus appears to be a demethylation, catalysed by trimethylamine *N*-oxide demethylase, yielding dimethylamine and formaldehyde.

P. M. gratefully acknowledges the award of a Science Research Council Studentship.

- Andrews, P. (1970). In *Methods of Biochemical Analysis*, vol. 18, p. 1. Ed. by Glick, D. New York: Interscience Publishers Inc.  
 Bystedt, J., Swenne, L. & Aas, H. W. (1959). *J. Sci. Fd Agric.* **10**, 301.  
 Nash, T. (1953). *Biochem. J.* **55**, 416.  
 Weber, K. & Osborn, M. (1969). *J. biol. Chem.* **244**, 4406.

### Configurational Dependencies of [ $^{19}\text{F}$ ]Fluorine Chemical Shifts and Coupling Constants in Fluoromonosaccharides

By P. W. KENT, R. A. DWEK and N. F. TAYLOR. (*Department of Biochemistry, University of Oxford, Oxford OX1 3QU, and Department of Biological Chemistry, University of Bath, Bath BA2 7AY, U.K.*)

In general, chemical shifts and coupling constants for  $^{19}\text{F}$  are of an order of magnitude greater